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ON PATENTS AND TRADEMARKS

(12) SPECIFICATION OF INVENTION
TO RUSSIAN FEDERATION PATENTS

METHOD OF PREPARING GEL-LIKE MATERIAL FOR PLASTICS OF SOFT TISSUES

ABSTRACT

FIELD: medicinal polymers. SUBSTANCE: gel-like material containing 1.0- 8.0 wt % of acrylamide/methylene-bis-acrylamide copolymer (from 100:0.5 to 100:5.0) and 92.0-99.0 wt % of weakly alkaline water with pH 6.9- 8.5, level of permanganate oxidability up to 1 mgO/l and level of bromination capacity not higher than 3.0 mgBr/l is prepared by copolymerization of acrylamide with methylene-bis-acrylamide in aqueous medium at pH 9.0-9.5 in presence of peroxide polymerization initiator. Reaction mixture is incubated for 2 to 24 hr at 20 to 90 C and then 2-4 hr at 100-105 C. Upon implantation of this material, there is lack of marked inflammatory response and, in later phases, sclerotic phenomena and intergrowth of implant are not observed, while homogeneous structure is preserved. EFFECT: improved biological compatibility. 3 cl, 1 dwg

DESCTRIPTION

The invention relates to medicine and is intended for the use in surgical practice for plastics of soft tissues.

The problem of preparing artificial materials for substitution of soft tissues in cases of breast gland, muscle tissue and subcutaneous tissue plastics has been on agenda in medical practice for a long time already, the interest is in rather cheap and simple in preparation materials having the required physical and chemical properties (definite density and chemical inertness, shrinkability or swellability after placement in the organism) and chemical properties (biological passivity, in particular, lack of rejection reaction or any other tissue reaction). On top of that the material should have the form suitable for implantation into the gland or muscle tissue with minimal traumas for patient's organism.

Known in the art is the material (US Patent No.5,282,857) in the form of an aqueous gel comprising at least 70% of cellulose derivatives, such as methylcellulose, ethyl cellulose, hydroxyethylcellulose and other derivatives, said gel is suggested for prosthetics of breast or ovaries soft tissues. However, substantial amount (at least 70%) of the synthetic material in the gel does not permit to apply it in great volumes (more than 5-10 ml), since according to the data obtained in Moscow Medical Academy named after I.M.Sechenov (MMA hereafter) the cellulose-based polymer materials cause inflammatory sclerotic reaction (See Resolution No.1 by MMA).

Known is application of 3%-polyacryl amide gel (Inventor's Certificate USSR N 1697756) for replenishment of a vocal cord volume.

However, it is impossible to realize such application, since composition of the polymer and method of preparing gel are not known. At the same time it is known that biological activity and respectively biological passiveness of polyacryl amide gel completely depend on the composition of the monomers constituting the gel and method of preparation thereof.

The main task, for the solution of which the proposed invention is aimed, resides in the preparation of a material in the form of a gel made on the basis of an acrylamide copolymer and which is suitable, according to its biological and physicochemical properties, for use as a gel for the plastics of soft tissues.

The set task is resolved by that the material is provided in the form of a gel for the plastics of soft tissues containing a polyacrylamide and a liquid medium, wherein as said polyacrylamide it contains acrylamide and methylene-bis-acrylamide copolymer at a mass ratio 100 : 0.5 – 5.0; and, as said liquid medium, it contains weakly alkaline water at the following ratio of the components, in mass %:

Polyacrylamide	1.0 – 8.0
Water	92.0 – 99.0

and has a pH of 6.9 – 8.5, a level of permanganate oxidability not higher than 1.0 mg O per liter and a level of bromination not more than 3.0 mg Br per liter.

For plastics of subcutaneous connective tissue the gel-like material contains, preferably, the following ratio of the components, in mass %:

Polyacrylamide	1.5 – 2.5
Water	97.5 – 98.5

For plastics of muscle and glandular tissue the gel-like material contains, preferably, the following ratio of the components, in mass %:

Polyacrylamide	4.0 – 8.0
Water	92.0 – 96.0

The set task is also resolved in that a method is provided for preparing a water-containing polyacrylamide gel material for plastics of soft tissues in which, according to the invention, copolymerization of acrylamide with methylene-bis-acrylamide in water medium at pH 9.0 – 9.5 in the presence of a peroxide polymerization initiator is carried out; the reaction mixture being incubated at t = 20 – 90^{0}C for 2 – 24 hours and then at t = 100 – 105^{0}C for 2 – 4 hours.

Ammonium persulfate in an amount of 0.0006 – 0.03 mass % or hydrogen peroxide in an amount of 0.1 – 0.3 mass %, or both components at any ratio in amounts not exceeding the mentioned ones may be used as the polymerization initiator.

In order to provide pH of the reaction mixture, as water, used is water treated by a method of electrolysis.

The essence of the invention is illustrated by graphic materials (see the drawing) on which IR-spectrum (infra-red spectrum) of the proposed gel material made in the area of 4000-200 cm^{-1} is presented.

The essence of the invention consists in that, first of all, the components forming the polymer and their quantitative ratio, and the liquid medium and quantitative ratio of the polymer with the liquid medium providing the necessary density and the material consistence have been experimentally selected; secondly, the conditions of preparing the gel material which according to its physicochemical and biological properties is suitable for carrying out plastics of soft tissues have been also selected.

The reaction of copolymerization of acrylamide and methylene-bis-acrylamide is known (USSR, Author's Certificate No. SU 1105767). In the process of polymerization a cross-linked polymer is formed the structure of which depends on the synthesis conditions, quantitative ratio of reagents, qualitative composition of polymerization initiators and temperature conditions.

Due to the reaction mixture incubation in two stages – at first at more lower, and then at more higher temperatures, the proposed method permits to decrease the amount of unlinked amino groups (NH_2 radicals) in the polymer what is confirmed by IR-spectrum of the proposed gel-like material presented on the drawing. The material contains 5% of polyacryl amide, wherein for 100 parts by weight of acrylamide there are 2 parts by weight of methylene-bis-acrylamide, and 95% weakly alkaline water, the material having pH of 8.0, level of permanganate oxidization ability of 0.2 mg O per liter, bromination level of 0.5 mg

Br per liter and is obtained at the initial mixture incubation with t=60^{0}C within 12 hours, after that at t=100^{0}C for another 3 hours. It is evident from the above specter it has no bands of 1620cm^{-1}, responsible for deformation vibrations of NH radicals, and 3200 cm^{-1} and 3600 cm^{-1}, responsible for valence vibrations of said radicals. This fact indicates that in the polymer structure the content of NH_2 radicals is less than 1% of the functional groups total amount.

Besides that, it has been shown by pathomorphological studies (See Resolution No.2 by MMA) that the one-stage incubation of the reaction mixture only at t = 30 – 90^{0}C or only at t = 100 – 105^{0}C leads to the preparation of gel having a level of permanganate oxidability from 2.0 to 5.0 mg O per liter and a level of bromination from 3.0 to 5.0 mg Br per liter. When such a gel was administered to rats, the inflammation reaction and tissue sclerosis, as well as accelerated gel resorption were observed.

The proposed method permits also to exclude the stage of washing off the prepared material from toxic initial monomers, since the concentration of initial components and the conditions of polymerization permit to prepare gel containing no non-reacted monomers, what is confirmed by the results of the test of final product.

Known is the polyacryl amide gel (International Application WO 81/01290) for eye lens preparation, containing 11.0% wt. of co-polymer of acrylamide and methylene-bis-acrylamide at the ratio 100:2.26 and 89% wt. of physiological solution.

The method for preparing the above gel (International Application WO81/01290) consists in that acrylamide and methylene-bis-acrylamide dissolved in the physiological solution are polymerized in the presence of polymerization initiators, one of them being tetramethylethylenediamine, followed by final product washing from non-reacted monomers. The polymerization is conducted in one operation.

The resultant gel is not suitable for use as a soft tissue plastics material, since due to the one-step polymerization process it comprises free NH_2 radical, on which its biological activity depends, and the amount of which depends on the agent composition and temperature conditions of the polymerization process.

Moreover, the use of tetramethylenediamine as a polymerization initiator affects negatively the quality of gel as a material for tissue plastics, since NH_3 groups remain included in the polymer molecule structure, said groups also negatively affect the organism tissue reaction, giving rise to tissue fibrosis (see Resolution by MMA).

For preparation of the suggested gel the following ingredients are taken:

- acrylamide: C_3H_5NO, mol.weight 71.08, white crystalline odorless powder, melting temperature 84.5^{0}C; manufactured by Sigma (USA), suitable for bio-medicinal use;
- methylene-bis-acrylamide: $C_7H_{10}N_2O_2$, mol.weight 154.16, white crystalline odorless powder, melting temperature 185^{0}C; manufactured by Sigma (USA), also suitable for biological use;
- ammonium persulphate: $(NH_4)S_2O_8$ – mol.weight 228.19, colorless flat crystals; decomposition temperature 120^{0}C; manufactured by Sigma (USA);
- hydrogen peroxide: H_2O_2 – mol.weight 34.0; colorless liquid, density 1.465 at 0^{0}C; melting T –0.89^{0}C; manufactured by Sigma (USA).

Acrylamide and methylene-bis-acrylamide are taken as suitable for biological use and requiring no additional purification.

Water is purified by means of bi-distillation and then subjected to electrolysis, as described in detail in "Methodology instructions for preparation of electro-chemical activated solutions (neutral analyte) made on СТЭЛ-4М-О1 plant for the purposes of pre-sterilizing purification and sterilizing", Moscow, 1993.

The gel of the present invention is prepared as follows.

Bi-distilled water subjected to electrolysis at the voltage of 220V and the current of 6A and having pH of 9.0 – 9.5 after the electrolysis treatment is used for the preparation of the reaction mixture. Water solution of acrylamide and methylene-bis-acrylamide at mass ratio to each other of 100.5 : 0.5 – 5.5 is prepared, the total mass of the starting monomers in the solution being 1.0 – 8.0 %. By varying the amount of starting monomers in the solution, gels of different density and elasticity are obtained. Polymerization initiators, and namely, hydrogen peroxide in an amount of 0.1 – 0.3 mass %, or ammonium persulfate in an amount of 0.0006 – 0.03 mass %, or their mixture at any ratio and amount not exceeding the sum of their maximum values are introduced into the obtained solution. The ready-made reaction mixture is filtered through bactericide polymer filters (type F8273, product of Sigma (USA) having the pore size 0.45 mm CA/CN) and, in a stream of nitrogen, is poured into glass vials in a required volume. The vials are hermetically sealed and placed for incubation at t = 20 – 90^{0}C for 2 – 24 hours and then the temperature is increased to 100 – 105^{0}C and the incubation is further carried out for more 2 – 4 hours.

With the availability of hydrogen peroxide in the incubation medium said hydrogen peroxide transfers into water and ozone which sterilizes the final product. However, for the

safety, the prepared gel is subject to sterilization by autoclaving (t = 120^0C, p = 1.2 atm) for 30 minutes.

The following characteristics of the resultant material were tested: refraction index (according to the methods described in "Physical Chemistry Practicum", Moscow, 1974, pages 86-97);

pH, and permanganate oxidability level – according to the methods described in "Methodology instructions for sanitary and hygiene estimation of rubber and latex articles for medicinal use", Moscow, 1988, pages 18-19;

Bromination level – according to the methods described in "Collected works on methods of toxicological assay of polymer materials and articles based thereon for medicinal use", Moscow, USSR Ministry of Health, 1987, pages 27-29;

Content of acrylamide and methylene-bis-acrylamide monomers – according to the methods described in "Collected works on methods of toxicological assay of polymer materials and articles based thereon for medical use", Moscow, USSR Ministry of Health, 1987, pages 18-25.

The prepared material has the following physicochemical characteristics:

External appearance	Colorless gel
Index of refraction	1.328 – 1.360
Density	0.9 – 1.2 g/cm^3
pH	6.9 – 8.5
Content of acrylamide monomers	absent
Content of methylene-bis-acrylamide monomers	absent
Level of permanganate oxidability	0.2 – 1.0 mg O per liter
Level of bromination	not more than 3.0 mg Br per liter

The sanitary-chemical tests of the proposed material were carried out in the Scientific-Research Institute of Rubber and Latex Goods (NIIR), toxicological and patho-morphological assay in Moscow Medical Academy named after I.M.Sechenov and in All-Russia Scientific-Research Institute of medical equipment testing (VNIIIMT) according to the program elaborated by VNIIIMT. The tests have established that the material proposed for plastics of soft tissues does not cause tissue reaction, organism sensibilization; it is not mutagenous and does not cause dystrophic or necrotic changes; and it is recommended for endoprosthesis and for contour plastics (See Resolution No.3 by MMA).

The preparation of the proposed material is illustrated in the following Examples of the concrete embodiment.

EXAMPLE 1

For preparation of gel, 400 ml of bi-distilled water was taken treated by electrolysis at 220V and current of 6A as described in "Methodological instructions for preparation of electrochemical activated solutions (neutral analyte), prepared in СТЭЛ-4М-60-1 plant for the purposes of pre-sterilizing purification and sterilizing", Moscow, 1993, and having after treatment pH value of 9.0, and 20 g of acrylamide and 0.1 g of methylene-bis-acrylamide useful for biological purposes was dissolved therein. After that 0.04 g of ammonium persulphate and 2 ml of 30% hydrogen peroxide were introduced in the initial solution. The resultant mixture was filtered through a bactericide polymer filter of F8273 type with pore size of 0.45mm CA/CN, manufacturer Sigma (USA) and poured in nitrogen flow by 100 ml into glass flasks. The flasks were hermetically sealed and placed for incubation on a water bath at the temperature of 30^0C for 22 hours, after that the temperature was raised to 105^0C and incubated for another 2 hours.

The resultant gel was sterilized by autoclave method (t=120^0C, p=1.2 atm.) for 30 minutes.

The resultant material had the following physical and chemical characteristics:

Outer appearance	colorless gel
Refraction index	1.348
pH	7.2
Density	1.0 g/cm^3
Acrylamide monomers content	none
Methylene-bis-acrylamide monomers	none
Permanganate oxidability level	0.4 mg O per liter
Bromination level	0.1 mg Br per liter.

The so obtained material was implanted to Ms.L.patient, aged 55 in place of silicone prosthesis of "Dow Corning" company which 8 years back had been used for primary breast plastics and which had given rise to coarse fibrosis of both lactic glands. The operation of implanting the suggested material 200 ml per each gland) was conducted on 9th September 1995. The patient was supervised in post surgery period for 8 months with monthly examinations. No fibrosis recurrence was observed. Cosmetic effect the patient estimates as very good.

EXAMPLE 2

For preparation of gel, 1000 ml of bi-distilled water treated by electrolysis (as described in Example 1) and having pH value of 9.5 was taken and 16 g of acrylamide and 0.8 g of methylene-bis-acrylamide was dissolved therein. Then 10 ml of 30% hydrogen peroxide was introduced into the initial solution. The resultant mixture was filtered as described in Example 1, and poured in the nitrogen flow by 50 ml into glass flasks. The flasks were hermetically sealed and placed for incubation at t=30^{0}C for 2 hours, then the temperature was raised to 100^{0}C and the flasks were incubated for another 4 hours.

The resultant gel was sterilized as described in Example 1.

The resultant material had the following physical and chemical characteristics:

Outer appearance	colorless gel
Refraction index	11.334*
pH	8.3
Density	0.95 g/cm^3
Acrylamide monomers content	none
Methylene-bis-acrylamide monomers	none
Permanganate oxidability level	10.6 mg O per liter
Bromination level	0.15 mg Br per liter.

The so obtained material was used for subcutaneous cellular tissue plastic in case of face wrinkles removal. The gel was implanted to Ms.S., a patient of 47. The operation for wrinkle removal was conducted on 20th May 1995. The patient was under supervision during the after surgery period for 12 months with periodical examinations once in three months. No inflammation or allergy occurrences were observed. The patient estimated the cosmetic effect as very good.

EXAMPLE 3

For preparation of gel, 1000 ml of bi-distilled water treated by electrolysis was taken (as described in EXAMPLE 1) and having after treatment pH value of 9.3, and 80 g of acrylamide and 2.4 g of methylene-bis-acrylamide was dissolved therein. After that 0.03 g of ammonium persulphate was introduced. The resultant mixture was filtered as described in Example 1, and in the nitrogen flow poured by 100 ml into glass flasks. The flasks were hermetically sealed and placed for incubation at the temperature of 60^{0}C for 12 hours, after that the temperature was raised to 105^{0}C and the flasks were incubated for another 2 hours.

The resultant gel was sterilized by autoclave method as described in Example 1.

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* This seems to be an obvious misprint; evidently should it be 1.344

The resultant material had the following physical and chemical characteristics

Outer appearance	colorless gel
Refraction index	1.352
pH	8.0
Density	1.2 g/cm^3
Acrylamide monomers content	none
Methylene-bis-acrylamide monomers	none
Permanganate oxidability level	0.2 mg O per liter
Bromination level	0.05 mg Br per liter.

The so obtained material was used for musculus gastrocnemius plastics. The gel in the amount of 150 g per one muscle was implanted to Ms.S.patient, aged 47. The operation of implanting the suggested material was conducted on 20th May 1995. The patient was supervised in post surgery period for 12 months with examinations every three months. No inflammation occurrences or edema were observed.

Cosmetic effect the patient estimated as good.

EXAMPLE 4

For preparation of gel, 400 ml of bi-distilled water treated by electrolysis (as described in Example 1) having pH value of 9.5 was taken and 16 g of acrylamide and 0.064 g of methylene-bis-acrylamide was dissolved therein. Then 0.02 ml of ammonium persulphate and 1 ml of 30% hydrogen peroxide were introduced into the initial solution. The resultant mixture was filtered as described in Example 1, and poured in the nitrogen flow by 50 ml into glass flasks. The flasks were hermetically sealed and placed for incubation at t=50^{0}C for 16 hours, then the temperature was raised to 105^{0}C and the flasks were incubated for another 2 hours.

The resultant gel was sterilized as described in Example 1.

The resultant material had the following physical and chemical characteristics:

Outer appearance	colorless gel
Refraction index	1.348
pH	7.8
Density	1.0 g/cm^3
Acrylamide monomers content	none
Methylene-bis-acrylamide monomers	none
Permanganate oxidability level	0.3 mg O per liter
Bromination level	0.12 mg Br per liter.

The so obtained material was used for implantation to Ms.Sh.patient aged 26 in place of a silicone prosthesis of domestic make, which was used 3 years back for conducting primary breast plastics and after 7 months after surgery caused fibrosis of both lactic glands. The operation for removal of silicone prostheses was performed with open capsulotomia and delayed implantation, 180 g in each gland of the resultant material took place on 14th October 1995. After 3 months another 100 g of the same gel was implanted in each gland. The patient was supervised in the after surgery period for 7 months with examinations once in two months. No fibrosis recurrence was observed. The patient estimated the cosmetic effect as good.

Hence, the above Examples of concrete implementation prove simplicity of the suggested method, possibility of preparing the suggested material and possibility of its use for soft tissue plastic.

CLAIMS

1. A method of preparing a gel-like material for plastics of soft tissues by copolymerization of acrylamide and methyl-bis-acrylamide in an aqueous dispersed medium in the presence of a peroxide polymerization initiator with holding a reaction mixture during a period of time for cross-linking a copolymer, ***characterized*** in that the copolymerization is carried out when incubating the reaction mixture at t = 20 – 90^{0}C for 2 – 24 hours and then at t = 100 – 105^{0}C for 2 – 4 hours, ammonium persulfate and/or hydrogen peroxide in an amount of not more than 0.3 mass % being used as said initiator of copolymerization, and water subjected to electrolysis being used as said aqueous medium, the starting ratio of components of acrylamide and methylene-bis-acrylamide being 100 : 0.5 – 5.0 mass parts.

2. A method according to claim 1, ***characterized*** in that ammonium persulfate is used in an amount of 0.0006 – 0.03 mass %.

3. A method according to claim 1, ***characterized*** in that hydrogen peroxide is used in an amount of 0.1 – 0.3 mass %.